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Translocation in tomato

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TRANSLOCATION IN TOMATO

by

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TABLE OF CONTENTS

INTRODUCTION

Of all the facets of plant physiology that have been studied, none has proved more difficult or confusing than translocation in the phloem. Since the early 1900's, the mechanism involved in phloem transport has been a matter of controversy, and several theories have been advanced to explain it. Such processes as simple diffusion, mass flow of solutions, cytoplasmic streaming and activated diffusion have been suggested to explain the movement of assimilates in the phloem. At the present time there are still two main schools of thought on the subject. The supporters of the mass flow theory of Münch (40) suggest that phloem translocation occurs, from point of production of assimilates to points of utilization, by means of a purely physical process. Other workers feel that phloem translocation is an energy requiring process and is therefore dependent upon the respiration of living cells. This suggestion was first made by De Vries (17) and later supported by Curtis (13) and by Mason and Phillis (36). Experimental evidence has been advanced in support of each of these theories, but the data are conflicting and it is not yet possible to determine whether one or the other, or neither, of these mechanisms is concerned. It may well be, that assimilate movement occurs by means of a combination of mechanisms.

Of the many techniques used in studies of translocation,

the most popular are those in which a portion of the phloem of the experimental plant is completely removed, or is interrupted in some fashion. Both phloem destruction and high or low temperatures are used to interrupt the phloem.

It is the purpose of this work, to determine the effect of certain treatments on the movement and accumulation of both endogenous and externally applied materials. It is hoped that the results of these experiments may increase our understanding of the translocation mechanism and of the factors concerned in the direction of movement.

REVIEW OF LITERATURE

Temperature and Translocation

One of the earliest reports on the effects of temperature on translocation in the phloem was that of Child and Bellamy in 1919 (8). They reported that the chilling of bean plant stems to 3 to 5°C between the cotyledons and first pair of leaves forced the development of the buds in the axils of the cotyledons. The buds above the cooled portion of the stem were retarded in their growth for 2 to 3 days. The upper limit of effectiveness of the cold treatment was at 5 to 6°C at which temperatures the cotyledonary buds may or may not be forced. In the same paper Child and Bellamy also reported on the effect of chilling a section of runner of Saxifraga sormentosa L. Ordinarily, runners of this species attain a length of 20 to 30 cm before they produce a new aerial plant. Cooling a section of this runner to 3 to 4°C caused a new plant to be produced in 2 to 4 days, even though the runner length was less than normal. An interesting point to note was that the plant, once initiated, developed normally, thus indicating no reduction in food supply from the parent plant. However, it appeared that something, perhaps some growth promoting substance, was inhibited in its movement. The same workers (9) published in 1920 on the effect of cooling the petiole on leaf budding of Bryophyllum. They found that a

petiole temperature of 2.5 to 4°C, maintained for a few days, was effective in inducing the development of leaf buds. The movement of water to the leaf was not appreciably affected.

Curtis (14) in 1929 studied the effect of petiole temperatures on movement of sugars out of the leaves of Red Kidney beans. Rough starch tests on leaf discs showed consistently higher starch in the leaves with chilled petioles. Sugar and total carbohydrate determinations showed significantly higher carbohydrate concentrations in leaves with petiole chilled to 2 to 3°C. Chilling to 7 to 9°C did not give significant retardation of sugar transport from the leaves. Curtis also reported that stem temperatures of 2 to 5°C interfered with upward movement of solutes in these plants. He suggested a correlation between the effect of chilling on translocation and on protoplasmic streaming. Curtis pointed out that cytoplasmic streaming slows down and ceases in the same temperature range as transport (2 to 5°C). Curtis reported no wilting of plants even when stem temperatures were reduced to 2°C.

Curtis and Herty (15) studied the effect of petiole temperature on translocation from leaves of Red Kidney beans. Basing their conclusions on dry weight changes only, they reported that petiole temperatures between 0.5°C and 4.5°C greatly reduced transport of carbohydrates from leaves. However, transport was not completely stopped even at the lowest

temperature. Temperatures of 7 to 11°C resulted in distinctly more transport than the lower temperatures, but less than temperatures of 17 to 24°C. A significant amount of transport took place when entire plants were placed in a room at 0 to 2°C. Schumacher (46) has reported that fluorescein movement through sieve tubes was about 1 to 3 cm per hour when entire plants are held at 1 to 4°C, whereas at 20 to 30°C this dye moved 20 to 30 cm per hour.

In 1941, Borthwick et al. (7) showed that water at 25°C circulated around the petiole of a Biloxi soybean leaf had no effect on the development of flower primordia. However, water at 3°C reduced floral primordia development to one in ten plants, while at 6°C only three of eleven plants were affected. These experimenters also reported that the increase in length of the centre leaflet of the lower leaf in the bud was greater in the control plants than in those with 3°C petioles. This work suggests not only a temperature inhibition of the movement of some flowering substance but inhibition of the movement of carbohydrates also.

Went has made extensive studies on the growth of tomato plants under controlled conditions (51). In 1944 he reported that plants held at 26.5°C at night had higher sucrose concentrations in their leaves than plants held at 18 to 20°C. He concluded from this that translocation of sugars is small at 26.5°C and steadily increases as the temperature decreases

to 8°C. He failed to take into account, however, any effects that these temperatures may have had on respiration of the whole plant.

Hewitt and Curtis (22) studied the effect of temperature on loss of dry matter and carbohydrates from leaves of beans, milkweed and tomato. Plants were held at 4°C, 10°C, 20°C, 30°C and 40°C for 13 hours. Corrections were made for respiration losses. It was shown that translocation from leaves increased progressively from 4°C to 20°C. For milkweed, loss at 30°C was higher than at 20°C but for bean and tomato the losses were about the same at these temperatures. Losses were greater in all cases at 30°C than at 10°C or 4°C. Translocational losses were greater at 40°C than at 4°C or 10°C but less than at 20 to 30°C. The authors state that the reduction in loss at the high temperature was due to high respiration and lack of carbohydrates to transport, rather than to injury of the transport mechanism. The greatest gain in carbohydrates in the roots of bean plants and the greatest loss in the stem both occurred at 20°C.

In 1949 Went and Hull (52) studied the effect of stem temperature on translocation of carbohydrates in tomato plants. Plants were selected for experimentation when they were 60 to 90 cm. tall. The leaves were pinched off the lower stem four days before the experiment was carried out. A copper temperature control collar 22 cm. long was placed around the lower

stem and the openings at the top and bottom were plugged with cotton wool. Water at various temperatures was pumped through a copper tube passing longitudinally up one side and down the other side of the collar. Two of the leaves on the plant were placed in a container of 7 per cent sucrose solution. The plant was decapitated and a "bleedometer" was attached to the cut end. The temperature change over the experiment was held to less than 1°C. Control plants had no water pumped through the collar and were at room temperature (23.0 ± 1°C). Following each experiment the roots were removed and dried for sugar analysis, using the ceric sulphate method.

When leaves were immersed in pure water there were no differences in bleeding rates between 1°C and 23°C stems. When the leaves were immersed in the 7 per cent sucrose solution, plants with 1°C stems began increased bleeding from the cut stump before the plants with 23°C stems. The authors suggest that as a result of sucrose addition to the leaves, a sudden increase in root activity occurs approximately ten hours later. This was measured as an increased rate of bleeding. In four of five groups of plants there was an increase in bleeding rate earlier in the 1 to 15°C stems than in the 23 to 24°C stems. Also, cooled plants bled more profusely. The authors explain these results by saying "translocation is enhanced at the lower temperatures". It is interesting to note however, that the warm stem plants had higher concentra-

tions of sucrose in the roots than the cold stem plants in all tests but one. This fact was overlooked in the discussion of mechanisms and explained on the basis that the roots were harvested several hours after the experiment.

Hull (24) publishing alone, reported experiments similar to those conducted by Went and Hull (52) and just discussed. A similar type of cooling collar was used to control tomato stem temperatures and sugar beet petiole temperatures. Hull studied carbohydrate accumulations in the roots and stems of tomato plants and the distribution of C^{14} after feeding labelled sucrose to a leaf. Hull states that his experiments indicated an equal or greater translocation at low temperatures. However, the published radioautographs do not support this statement although the sucrose data, unlike those of Went and Hull (52), do support such a statement.

Swanson and Böhning (48) have reported on the use of stem elongation as an index of the effect of temperature on translocation of sugars. Experiments were conducted with two varieties of beans, in which the plants were treated after exposure to a 24 to 48 hour dark period. Temperature jackets were made of masonite and heating of petioles above room temperature was accomplished with nichrome heating wires. Other masonite boxes were refrigerated to approximately 4°C. Temperatures varied only 1°C. One leaf of each plant was rolled and placed in a solution of 0.75 M sucrose, 0.025 % sulfanila-

mide and 0.2 % Tween 20. Final growth measurements were made after 4 or 5 days in the dark. Maximum rates of transport occurred at petiole temperatures between 20°C and 30°C. At petiole temperatures both above and below these, elongation rates, and presumably translocation, were reduced. Transport was reduced 50 to 100 per cent at temperatures of 5 to 7.5°C and 40 to 42°C. These results are similar to those reported by other workers (5, 22) for temperatures between 20°C and 30°C. Swanson and Böhning found that the retardation effect of lower temperature on translocation was reduced after several days, whereas the high temperature retardation effect increased over the same period.

Kendall (27), in 1952, reported a very interesting study on the effect of intermittently varied petiole temperatures on sugar transport. He showed that a constant petiole temperature of 25°C allowed the greatest stem elongation in an 81-hour experiment. Least translocation, as measured by stem elongation, occurred through petioles the temperatures of which were alternated between 5°C and 25°C (12 hours and 12 hours). An intermediate amount of transport occurred with alternate petiole temperatures of 25°C and 40°C. Alternating temperatures of 5°C and 25°C had greater retarding effects than continuous low temperatures. Kendall reported that the plants showed a physiological adjustment to low temperatures but this adjustment was slower with intermittent temperatures.

Kendall suggested that greater transport at alternating high and medium temperatures may be due to less protoplasmic injury as a result of shorter exposures to the high temperatures.

Böhning et al. (4) showed by the use of temperature control jackets, that the hypocotyl temperature of bean plants markedly affected the elongation of the stem. Accepting elongation of the stem as an index of translocation (48), they showed that maximum translocation occurred at 33°C. They concluded, as did Day (16), that interference with the phloem at any point along the stem or petiole affects transport at all other points. Such a view, they say, is compatible with the idea that translocation in the phloem is mainly by mass flow.

The advent of radioisotopic techniques has provided a useful tool in studies on translocation as affected by temperature. Vernon and Aronoff (50) in 1952 showed that, contrary to Hull's results (24), chilling the stem of a soybean plant resulted in a great reduction in translocation. Ice and water were packed into a glass cylinder sealed around the stem of a soybean plant. Water at 30°C was placed around the stem of a similar plant. Carbon dioxide labelled with C^{14} was applied to a leaf and after 20 minutes the position of the radioactivity front in the stem was determined. It was found that the front was 12 cm. down the stem in the chilled plant (8 cm. below the water level)! In the 30°C stem plant the front was 28 cm. down the stem.

Studying the translocation of foliar applications of P^{32} and other radioisotopes, Swanson and Whitney (49) have shown marked inhibition of transport by low petiole temperatures. They reported an 85 per cent inhibition at 5°C, with optimum transport occurring at 30°C. Of interest also is the fact that P^{32}, Cs^{137} and K^{42} showed apparently independent rates of movement.

Hodgson (23) has made a study of temperatures in the range 37.5 to 56.5°C in relation to the effect on transport in Black Valentine beans. Plants were fed $C^{14}O_2$ by the leaf-cup method or randomly labelled C^{14}-sucrose by the leaf-flap method. Phosphorus 32 was supplied by either spot application or flap injection. A petiole temperature of 42°C gave the highest activity levels in stems relative to controls. Flap injected P^{32} moved from leaves faster and in greater quantities than spot applied. The author thought that the flap injected isotope passed through the petiole in the phloem but was less temperature sensitive than spot applied. Labelled sucrose also appeared to be more temperature sensitive than $C^{14}O_2$ photosynthate.

Mortimer (39) has recently studied the effect of petiole temperature on the movement of $C^{14}O_2$ photosynthate out of sugar beet leaves. He found that the apparent velocity of transport is not changed by chilling the petiole to 10°C or 20°C or warming it to 33°C. Chilling a small section of the

petiole to 2°C blocks movement past the chilled region. Further, the $C^{14}O_2$ photosynthate accumulated above the chilled region in the midrib of the leaf. Apparently some temperature effect is moving up the petiole from the low temperature block, since a warm jacket applied immediately above the cold zone caused the photosynthate to accumulate just above the cold jacket.

Phloem Destruction and Translocation

Probably the most widely used technique in phloem translocation studies has been that of removal or killing of the phloem. In those species with a definite bark such as cotton or privet, the phloem can be interrupted by removal of a ring of bark from the stem. In those plants, such as tomato or bean, which have no definite bark, interruption of the phloem has been accomplished by killing the living tissues in a small portion of the stem with a jet of steam or a ring of hot paraffin.

Beginning in 1920 Curtis published a series of papers dealing with the effect of ringing on translocation in such plants as privet, peach, lilac and apple. He attempted to determine the tissue, phloem or xylem, which transports mineral nutrients from the roots to the tops of the plant. In 1923 Curtis offered evidence that nitrogen is moved upward in the phloem (12). He showed that when sodium nitrate was added

to the soil, the nitrogen contents of the leaves of unringed plants increased to a much greater degree than did those leaves from ringed stems. Curtis felt that the results were not due to lessened transpiration or to changes in the carbohydrate contents of the ringed stems. Phillis and Mason (43) have criticized this conclusion. In other experiments, shoots attached to the parent plant by xylem alone failed to grow, while those attached by phloem alone grew almost as well as intact plants (13).

Earlier, Curtis had published results of experiments concerning carbohydrate changes in ringed plants (11). He found that ringed, defoliated shoots ceased growth after a short period of time, while ringed stems, not defoliated, showed considerable growth. When dormant stems were ringed, the growth above this ring ceased soon after the starch supply was depleted. Curtis felt that carbohydrates stored below the ring could not be moved in the xylem. They must be transported radially to the phloem and will be carried downward if there is no second ring below.

In a series of classical papers Mason and Maskell reported on their comprehensive studies on translocation in the cotton plant (33, 34, 35). Their general conclusions were that ringing the cotton plant caused phosphorus, potassium, nitrogen and carbohydrates to accumulate above the ring and to decrease below the ring. Nitrogen and ash constituents as-

cended the stem mainly via the wood, and were reexported from the leaves via the phloem. The downward movement of phosphorus could be reversed by reversing the relative positions of the leafy and fruiting regions of the stem. Mason and Maskell (35) further suggested that minerals are released from the phloem to the xylem, whereupon they may reascend the plant, setting up a form of circulation. This idea has been supported by others (2, 25, 26).

Loomis in 1935 (29), showed that phloem rings temporarily checked the movement of carbohydrates and organic nitrogen in apple, poplar and box elder. It is interesting however, that after periods of from 4 to 6 weeks the ringing effect seemed to disappear, Loomis suggested this was due to the increased permeability of the cells of the ringed segment, and the loss of organic materials through these cells into the xylem. He concluded from his work that nitrogen is synthesized to organic forms in the roots, and is normally moved upward in the phloem. This upward movement of nitrogen and the downward transport of sugars from the leaves can be checked by ringing.

Using a double ring technique on raspberries Engard has made some interesting observations regarding the translocation of sugars and nitrogen (18, 19). He made two rings on a raspberry cane, one 15 cm. above the ground and another 15 cm. above the first. After a period of time various portions of the plants were analyzed for sugars and nitrogen. He found

that total carbohydrates accumulated above the rings and decreased between and below them. Both sucrose and reducing sugars followed this pattern with sucrose making the larger increase. He concluded from this that sucrose moved upward as well as downward in the phloem. Unlike Maskell and Mason (33), Engard did not find an accumulation of nitrogen either above or below the rings. He concluded that there was no definite upward or downward movement of nitrogen in the phloem, and, that nitrate is the important form of translocated nitrogen in raspberry.

Stout and Hoagland (47) concluded from root feeding experiments with radioisotopes of phosphorus, potassium, sodium and bromine that the xylem is the path upward for nutrient elements. They found that these elements move very slowly through strips of bark. They reported that there is rapid radial movement of these isotopes from wood to bark when these tissues are in contact. Rabideau and Burr (45), similarly, showed that P^{32} fed to roots would pass upward past killed areas of bean stem. Leaf fed C^{13} on the contrary would not pass downward past a killed section of stem.

Colwell (10) in 1942, reported on experiments in which he interrupted the phloem of squash plant stems by scalding a short section in boiling water. He found that such a treatment to the petiole of a leaf prevented the export of P^{32} out of this leaf, provided that the P^{32} solution was not applied

to a large area of the leaf. Colwell's studies showed that if transport from the leaf is restricted to the phloem, P^{32} movement is predominantly in the direction of food movement. When P^{32} is applied to a lower leaf, it moves mostly to the roots. If applied to a middle leaf P^{32} moves to the roots and shoot, while if applied to an upper leaf it moves to the shoot tip. Colwell supported the work of Mason and Maskell regarding radial movement of mineral elements from phloem to xylem.

Bonner (6), studied the effect of girdling tomato stems with superheated steam on the accumulation of various substances. He found that such compounds as thiamine, pyridoxine, riboflavin and sucrose accumulated in the region of the girdle. These materials were accumulated at varying rates and to varying extents. After one and one-half days there was three times as much sucrose accumulated above the girdle as there was in the normal stem.

Swanson and Whitney (49) found that steaming the petiole of bean leaves prevented the movement of P^{32}, K^{42}, Ca^{45} and Cs^{137} out of these leaves. These results are the same as those obtained by Colwell (10) on squash plants. However, contradictory results have been reported by Nelson et al. (42). They have reported that part of C^{14}, fed as $C^{14}O_2$ to the primary leaf of a bean plant, was transported downward past a steam girdle but did not accumulate in the roots. They accepted this as evidence for translocation of organic materials through dead tissues.

MATERIALS AND METHODS

Introductory Experiments

Tomato plants (variety Rutgers) were grown in 4 inch pots, in the greenhouse, to a height of 20 to 24 inches. The plants were then removed from the pots, and, in order to accommodate the glass jacket which would subsequently be placed on them, the lower 7 or 8 inches of stem was stripped of all leaves. Also, to at least partially ensure that all plants were undergoing vigorous root growth, all secondary roots were cut away with a knife except for a 1 1/2 to 2 inch cube of roots and soil. These experimental plants were then repotted, watered and treated.

Temperature treatments were applied by the use of glass jackets which were secured with masking tape around the lower stem of the tomato plants. These jackets were in turn wrapped with a spongy, plastic, insulating material which was also secured with masking tape. Ice water was pumped through these jackets with a small centrifugal pump. The ice water reservoir consisted of an insulated, galvanized metal box having inside dimensions of 12" x 12" x 27". Inlet and outlet pipes on the box allowed the coolant to be pumped out of the bottom of the box, around the plant stems, and back into the top of the box. Water cooling was obtained by placing large (25 lb) blocks of ice in the box. Coolant temperatures were deter-

mined by thermometers placed just before the coolant entered the jacket around the first plant and just after it left the last jacket in the series. Actual stem temperatures were determined with thermocouples.

The glass jackets consisted of two halves, each of which was kidney-shape in cross section. The jackets were about 1 1/2 inches long and were fitted with an inlet or outlet tube at each end. The jackets were placed on the stems in such a manner that the stem was in the hollow portions of the two halves of the jacket. The two halves were then taped together. Rubber tubing connections were arranged so that the coolant passed into the bottom of one of the jacket halves, out the top of this half and into the bottom of the second half, etc.

All of these introductory experiments were carried out in the greenhouse under the existing light conditions. All experiments were continued for one week.

It is obvious from the above description that the temperature control that could be obtained in such a system was limited. Observations showed that the lowest coolant temperature obtained was around 3°C and this temperature varied between 3°C and about 5°C. In later experiments a temperature control bath which would maintain temperatures between -20°C and 70°C ± 0.02°C was used.

An attempt was made in the preliminary experiments to make various measurements that would be an index of transloca-

tion. These measurements included elongation of growing tips, growth of roots and sugar distribution throughout the plants. It was finally decided that such things as shoot growth and possibly root growth were not good indices of translocation since they involved phenomena other than translocation. In later experiments all the emphasis was placed on measurements of sugar concentrations throughout the plant as affected by the temperatures.

At the end of the experimental period (one week in all preliminary experiments) the tomato plants were removed from their pots, the roots were washed and samples were taken. Three samples were taken from each plant: (1) all secondary roots, (2) a section of stem below the glass jacket, and (3) a section of stem above the jacket, between the jacket and lowest leaf. The samples were weighed and stem sections were sliced into approximately 1/8 inch slices. All samples were then placed in 50 ml of boiling, 80 per cent ethanol for killing and extraction of alcohol soluble carbohydrates (31). Samples were stored in the alcohol for a minimum of two weeks, with periodic shaking, before sugar determinations were carried out.

Samples were prepared for sugar determination procedures in the following manner. A 25 ml aliquot of the alcohol extract was evaporated almost to dryness. About 25 ml of water was added and the sample was then treated with 5 drops of

saturated neutral lead acetate to clear it of interfering materials. The extract was then filtered and made to volume in a 100 ml volumetric flask containing 5 ml of dipotassium phosphate (K_2HPO_4, 125 gm/l) to precipitate any excess of lead. These flasks were then placed in the refrigerator until the lead phosphate precipitate had settled (4 - 6 hr).

Reducing sugar determinations were made with 5 ml of the cleared, deleaded extract using the ceric sulphate method of Hassid (20, 21). Five milliliters of alkaline potassium ferricyanide was added to the 5 ml of extract in an 8-inch test tube. This solution was then heated in a boiling water bath for 15 minutes. The tubes were then cooled in tap water for 4 minutes. After cooling, 5 ml of 5 N H_2SO_4 was added to the samples and they were titrated with ceric sulphate. Reducing sugars were estimated from a standard glucose curve. Standard glucose determinations were made each time that a set of unknowns was titrated. Results were expressed as milligrams of glucose per gram of tissue (fresh weight). Another series of 5 ml aliquots was hydrolyzed with yeast invertase. These were also titrated with ceric sulphate, and sucrose concentrations were determined by subtraction. Sucrose standards were titrated each time as before. Again, results were expressed as milligrams sucrose per gram of tissue (fresh weight).

It was felt that the determination of starch, in the

samples that had been obtained, might be of value in giving an idea of carbohydrate distributions, and also in reducing some of the variability encountered. In one experiment, therefore, starch determinations were carried out on the alcohol extract residue. The method used was that described by Powell (44), slightly modified in some respects. The residue was dried in a 65°C oven for 24 hours and ground to 40 mesh in a Wiley mill. Twenty-five milliliters of distilled water was added to the ground residue and the mixture was autoclaved at 15 pounds for 15 minutes. When the samples had cooled, one drop of amylase (0.1 g/100 ml of a commercial preparation) was added and hydrolysis was allowed to proceed overnight at room temperature. The samples were then cleared, filtered and deleaded as before. To a 5 ml aliquot of these samples was added 0.2 ml of concentrated HCl and this solution was autoclaved for one hour at 15 pounds. The HCl was neutralized with 40 per cent NaOH and reducing sugar determinations were made. Results were expressed as milligrams of starch per gram of tissue (fresh weight).

Later Carbohydrate Distribution Experiments

A later series of experiments was conducted in approximately the same manner as that described for the introductory work. However, some changes in procedure were made.

Twelve tomato plants were removed from pots and pruned as

described previously. The plants were then repotted, watered and left in the greenhouse for one week to allow them to recover partially from the severe pruning. At the end of this period the plants were placed in the dark for 48 hours to reduce the sugar content of the plants to a lower and perhaps more uniform level. A similar technique has been used by other workers (4, 22). The plants were taken from the dark and set up on a laboratory table under about 800 foot-candles of artificial light. They remained there for the duration of the experiment. The artificial light was supplied by a bank of lights consisting of 6 - 40 watt fluorescent tubes and 9 - 50 watt incandescent bulbs.

Glass jackets, 4 inches in length, were placed on six of the tomato plants, while the stems of the other six plants were left bare. In one experiment these control plants were fitted with the jackets used in the introductory experiments through which water at room temperature was pumped. It was found that this water temperature varied as much as the room temperature, and therefore, in later experiments no jackets were used on the control plants.

The first of these carbohydrate distribution experiments were carried out with the stem temperatures of the treated plants at 3°C and 45°C for one week. Two subsequent experiments were performed with stem temperatures of 3, 6, 11, 16, 21, 25, 29, 37 and 45°C for 32 hours. The shorter time period

was used to overcome differences in root growth and to measure only carbohydrate accumulations. At each temperature, six plants were treated and six plants were maintained as controls at room temperature. Room temperature varied from 25°C to 32°C.

At the end of the specified times, the plants were removed from the apparatus and samples were obtained as described earlier. In these experiments, however, the samples taken included the roots plus a short basal stem portion, the section of stem covered by the jacket and the stem section between the jacket and the lowest leaf. Carbohydrate concentrations were determined and expressed as before. Starch concentrations were determined only in the first of the 32 hour experiments.

Radioisotope Experiments

Temperature experiments

In these experiments the tomato plants were prepared for experimentation as described previously. Twelve plants were root pruned, allowed one week for regrowth, darkened for 48 hours and set up on the temperature control apparatus. After 12 hours pretreatment at the desired temperature the lights were turned on and 15 μc of P^{32} in 40 μl (H_3PO_4, pH about 3) plus 0.2 % Tween 20 was spotted on the terminal leaflet of the lowest leaf left on the plant. The solution was applied with

a 50 µl microsyringe. The time allowed for absorption and translocation was 6 hours. In two experiments the temperature jackets were placed below the P^{32} treated leaf, and stem temperature of 0.5, 3, 6, 11, 16, 21 and 25°C were employed. In two other experiments the jacket was placed above the treated leaf and only the lowest temperature of 0.5°C was used.

After the 6-hour experimental period the plants were harvested, the treated leaf was discarded and the plants were divided into samples. These samples included: (1) the roots and basal portion of the stem; (2) the section of stem covered by the jacket; (3) the section of stem between the jacket and the treated leaf and (4) the tops. In experiments where the jacket was below the treated leaf, sample (3) was below that leaf, while sample (3) was above the treated leaf when the jacket was above the leaf.

After the plants were harvested the roots were washed and all samples were dried at 65°C for 24 hours. The samples were then ground to 20 mesh in a Wiley mill and pressed into a cylindrical wafer 5 mm thick and 20 mm in diameter. The total weight of the wafer was kept constant at 1.5 g through the use of filler tissue previously dried and ground. The pressing was done in a hydraulic press at a pressure of 14,000 pounds per square inch for 3 minutes. The above procedure provided P^{32} samples of "infinite" thickness which were then counted, using a specially constructed holder and spring mechanism to

hold the briquets. Such an apparatus provided constant geometry for counting the samples. The method described above is a modification (37) of one developed by Mackenzie and Dean (32). The scale used in counting was a Nuclear Chicago Model 161 Binary Scaler. The tube was a Tracer Lab TGC-1 with a 2.5 mg/cm^2 mica window. All samples were counted to about 1000 counts unless they were extremely high or low in activity.

It is obvious that absolute counts were not obtained, but since this was a comparative study absolute counts were not required. Results were expressed in several ways including counts per minute, specific activity, percentage of control and percentage of the total P^{32} moved out of the treated leaf.

Steaming experiments

In order to compare the effect of low stem temperature on translocation with another type of treatment, several experiments were conducted in which the phloem of the tomato plants was interrupted by killing a 15 to 20 mm section of the stem with steam. Plants were pretreated as before except that no 48 hour dark treatment was used. It was felt that if the amount of P^{32} movement was dependent on the sugar content of the treated leaf, it would be better to have the plants at a higher sugar level. Plants were removed from the greenhouse to the laboratory about 15 hours before treatment with P^{32}. At that time they were staked up and a segment of stem was

killed with a jet of steam. The amount of P^{32} applied, the manner in which it was applied and the time allowed for translocation were as described in the temperature experiments.

Samples were collected and treated as described above except that in these experiments an extra sample, the killed stem section, was collected. This section was ground, pelleted and counted separately.

Two experiments in which the six treated plants were steamed below the P^{32} treated leaf, and two experiments where the stem was girdled above the treated leaf were carried out. Results were expressed as described above.

Results of experiments on the effects of low stem temperature on P^{32} transport and distribution and those from steaming experiments suggested that valuable information might be gained from experiments involving both stem cooling and steaming. Therefore, four experiments in which tomato stems were chilled to 0.5°C above the P^{32} treated leaf and steamed below the leaf were conducted. All other conditions were as described previously for the steaming experiments.

RESULTS

Introductory Experiments

Results of the introductory experiments are given in Tables 1 and 2. The data in Table 1 show that the differences in root weight between cold-stem and control plants were large and significant near the 1 per cent level. No significant differences were found in the sugar concentrations in the roots of the two lots of plants. In two experiments the cold-stem plants had higher sugar concentrations in the roots, while in the other two experiments this situation was reversed. Starch determinations, which were carried out only in Experiment 2, indicated that there were no differences between cold-stem and control roots.

Because the root systems of the cold-stem plants were smaller, the total translocation of sugar to the roots was significantly reduced. The fact that there was a slight, but nonsignificant increase in sugar concentration in the cold-stem plants, indicates, however, that a sugar deficiency was not the direct cause of the reduced root growth. The results suggest that the translocation of some other essential growth factor was more severely limited in these week-long experiments than was the translocation of sugar.

It is interesting to note the similarity in the results of the present experiments with those of Child and Bellamy

Table 1. The effect of a stem temperature, below the lowest leaf, of about 5°C on the fresh weight and accumulation of carbohydrates in tomato roots after one week. (Avg. of 6 plants per treatment.)

Expt. No.	Treatment	Fresh wt. roots (g)	Mg carbohydrate/g fresh wt. Reducing sugars	Sucrose	Starch	Total
1	Cold stem	1.04	7.45	--	--	7.45[a]
2	"	2.97	2.59	0.66	1.08	4.33[b]
3	"	0.78	9.13	6.33	--	15.46
4	"	0.89	5.37	1.77	--	7.14
		5.68	24.54	8.76	1.08	34.38
1	Control	2.97	5.71	--	--	5.71
2	"	4.91	7.21	2.78	1.61	11.60
3	"	1.71	2.35	1.48	--	3.83
4	"	3.29	3.45	1.41	--	4.86
		12.88	18.72	5.67	1.61	26.00

Analysis of Variance of Root Weights

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	$F_{.05}$	$F_{.01}$
Between treatments	1	38.27	38.27	33.57	10.13	34.12
Between experiments	3	47.75	15.92	13.96	9.28	29.46
Error (TxE)	3	3.43	1.14			
Plants within treatments	40	35.62	0.89			
Total	47	125.07	2.66			

[a]Only reducing sugars determined in Experiment 1.

[b]Starch determined only in Experiment 2.

Table 2. The effect of a stem temperature, below the lowest leaf, of about 5°C on the distribution of carbohydrates in tomato stems after 1 week. (Avg. of 6 plants per treatment.)

Expt. No.	Treatment	Stem section	Fresh wt. stems (g)	Mg carbohydrate/g fresh wt. Reducing sugars	Sucrose	Starch	Total
2	Cold stem	Above	2.79	18.21	2.79	6.43	27.43[a]
3	"	jacket	0.46	11.99	6.00	--	17.99
4	"		1.76	12.30	15.32	--	27.62
			5.01	42.50	24.11	6.43	73.04
2	"	Below	2.56	15.46	1.18	3.47	20.11
3	"	jacket	0.51	10.20	3.31	--	13.51
4	"		1.41	11.06	8.32	--	19.38
			4.48	36.72	12.81	3.47	53.00
2	Control	Above	2.55	15.89	1.90	3.99	21.78
3	"	jacket	0.45	4.95	2.33	--	7.28
4	"		2.26	9.78	13.34	--	23.12
			5.26	30.62	17.57	3.99	52.18
2	"	Below	2.48	13.03	3.73	3.85	20.61
3	"	jacket	0.47	3.78	1.61	--	5.39
4	"		1.60	8.89	10.31	--	19.20
			4.55	25.70	15.65	3.85	45.20

[a]Starch determined only in Experiment 2.

(8). They reported that cooling a section of runner of Saxifraga sormentosa L. to 2 - 3°C forced the formation of a new aerial plant. Apparently some substance required for continued growth of the runner was being inhibited in its movement by the cold treatment.

The effect of a 5°C stem temperature on the distribution and accumulation of carbohydrates in the stems is shown in Table 2. The differences in sugar concentrations in the stems above the jacket and the stems below the jacket in the cold-stem plants were significantly higher than the differences in the control plants. A paired sample technique and the "t-test" were used in the analysis. Although it was determined in only one experiment, the data show that starch also accumulated above the cold jacket. These results show that a 5°C stem temperature reduced the movement of sugars to the roots and caused an accumulation of sugars above the cold region.

In contrast to these results Hull (24), working also with tomato, reported that he could detect no sugar accumulation above a 1 - 3°C stem collar. In fact, Hull reported lower sugar concentrations above a cold jacket than in the comparable stem position in control plants.

Later Carbohydrate Distribution Experiments

When employing techniques involving the use of cooling or heating jackets on the stems of plants, one must consider how far up or down the stem the cooling or heating effect

occurs. It has been suggested by Zimmerman (53), that the effect of low temperatures on translocation is mainly through the reduction in temperature in the top portions of the plant.

Figure 1 shows the reduction in tomato stem temperature above the jacket when the stem covered by the jacket was at 0.5°C. It is obvious that there was no effective reduction in stem temperature over 3 cm above the jacket. At points 5 cm above the jacket, or 3 cm below the jacket, there were no differences between treated and control plants. Since the jackets were never placed less than 3 cm below the treated leaf, it is fairly clear that there was no reduction in stem temperature at the node or in the petiole of the treated leaf. Mortimer (39) has stated that he could detect no reduction in temperature in the mid-rib of sugar beet leaves when a segment of the petiole was chilled. However, he felt that there was some effect in the leaf, since sugars accumulated in the mid-rib rather than in the petiole above the jacket. When a heating jacket was placed immediately above the cooling jacket, the sugars then accumulated above the cold jacket in the warmed zone.

One experiment was conducted in which the stem temperature of the treated plants was maintained at 45°C for 1 week. The results of this experiment are shown in Table 3. The total sugar concentration in the roots of the treated plants was reduced 75 per cent by the warm stem treatment. Root

Figure 1. Stem temperature of tomato as a function of distance above a cooling jacket. The stem temperature beneath the jacket was 0.5°C.

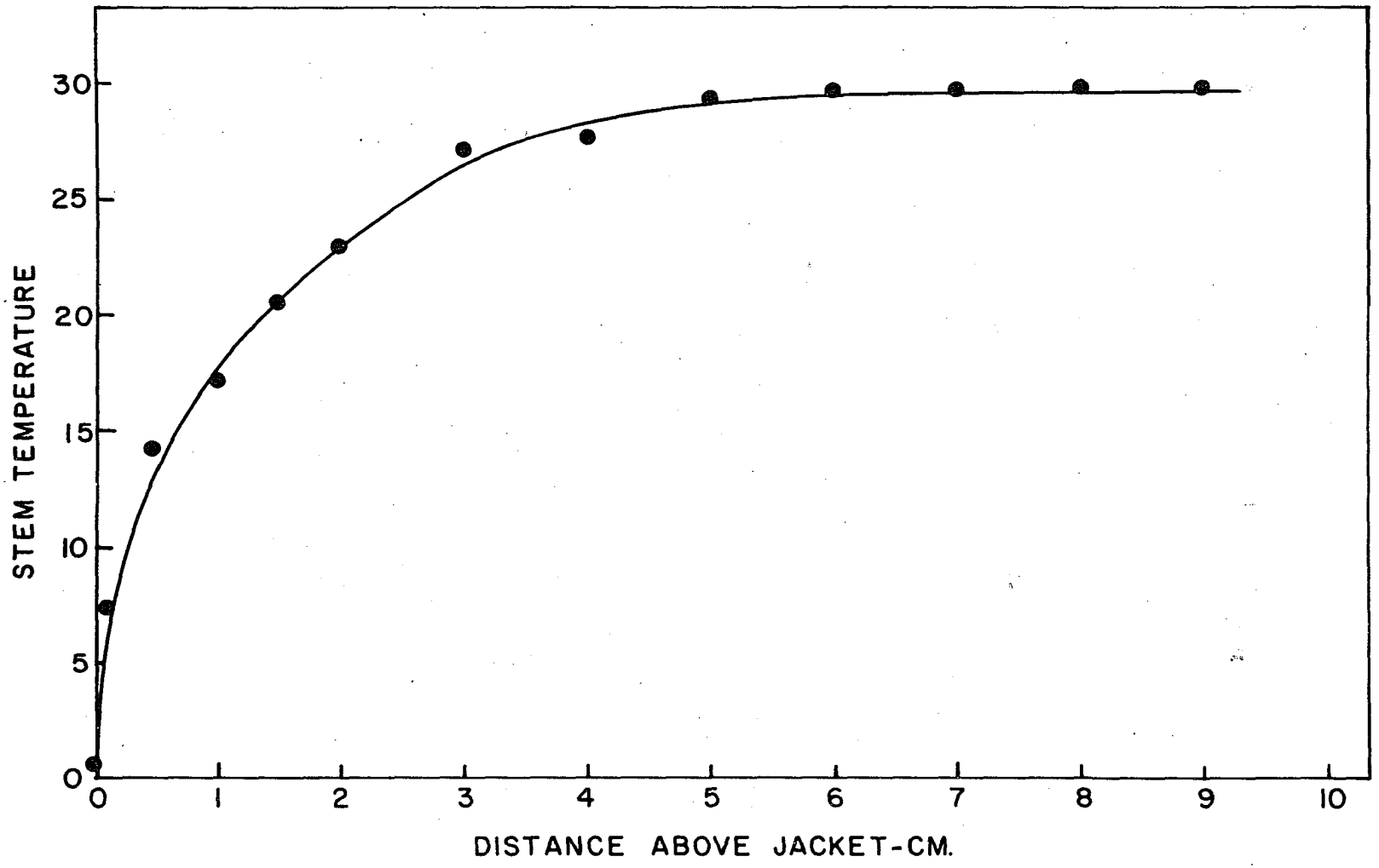

STEM TEMPERATURE
0
5
10
15
20
25
30
0
1
2
3
4
5
6
7
8
9
10
DISTANCE ABOVE JACKET-CM.

Table 3. The effect of a stem temperature, below the lowest leaf, of 45°C on the fresh weight and accumulation of sugars in tomato plants after one week. (Avg. of 6 plants per treatment.)

Treatment	Plant part	Fresh wt. (g)	Mg sugar/g fresh wt. Reducing sugar	Sucrose	Total
Warm stem	Roots	1.38	1.41	0	1.41
"	Stem covered by jacket	0.69	5.99	0.43	6.42
"	Stem above jacket	1.35	16.65	7.02	23.67
Control	Roots	2.52	3.13	2.48	5.61
"	Stem covered by jacket	0.68	7.69	9.44	17.13
"	Stem above jacket	1.29	11.48	6.28	17.76

weights in the same plants were reduced 50 per cent. Sugar concentrations in the jacketed area of the control plants were nearly three times those in the treated plants. As in the one week, cold-stem experiments, there was an accumulation above the jacket in the treated plants. It is interesting that in the roots of the six warm-stem plants, no sucrose could be detected.

A comparison of the data presented in Tables 1 and 3

shows that a temperature of 45°C had a greater effect on sugar movement to the roots than a temperature of 5°C. These results support the theory of Swanson and Böhning (48) who suggested that the effect of high temperature on translocation increased over a period of time, while that of low temperature decreased over the same period.

Because of the confounding of growth and translocation effects in the one-week experiments, further tests of sugar movement were limited to an experimental time of 32 hours. Also, the ice-water tanks used in the preliminary experiments were replaced by a thermostatically controlled temperature bath.

Data on the effect of stem temperature on carbohydrate distribution after 32 hours are presented in Tables 4 - 9. Two experiments of this type were conducted and the average results are given in Figures 2 and 3. Since it was found that starch concentrations did not affect the general results of these experiments, starch was not included in the calculations for Figures 2 and 3. Results in Figure 2 are expressed as the total sugar concentration in the treated plants as a percentage of control plants. Figure 3 is a plot of the ratio of sugar concentrations in the stem above the jacket and in the stem covered by the jacket, versus the stem temperature of the treated plants.

In Figure 2 it can be seen that reductions in root sugars

Table 4. The first experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of carbohydrates in the roots of tomato after 32 hours. (Avg. of 6 plants per treatment.)

		Mg carbohydrate/g fresh wt.			
Temperature, °C	Treatment	Reducing sugars	Sucrose	Starch	Total
3	Treated	1.54	1.10	6.70	9.34
	Control	4.02	3.92	11.16	19.10
6	Treated	1.62	1.06	9.28	11.96
	Control	2.21	1.71	11.67	15.59
11	Treated	1.90	0.64	11.17	13.71
	Control	2.18	0.42	12.71	15.31
16	Treated	10.96	5.59	2.29	18.84
	Control	8.37	4.66	2.70	15.73
21	Treated	6.79	3.15	1.69	11.63
	Control	6.36	3.80	1.45	11.61
25	Treated	4.61	3.56	1.87	10.04
	Control	5.63	3.96	1.88	11.47
29	Treated	2.56	1.74	10.01	14.31
	Control	3.12	1.64	11.23	15.99
37	Treated	4.84	2.85	13.27	20.96
	Control	5.10	3.06	14.16	22.32
45	Treated	3.54	0.46	10.96	14.96
	Control	5.23	3.16	17.56	25.95

occurred at both low and high stem temperatures. Root sugars were low from 0.5°C to 6°C and then increased rapidly to control levels at 11°C. Root sugars decreased very quickly above 37°C to about 55 per cent of the controls at 45°C. It is rather difficult to comment on the sugar levels occurring in

Table 5. The first experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of carbohydrates in the stem of tomato covered by the jacket after 32 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Mg carbohydrate/g fresh wt. Reducing sugars	Sucrose	Starch	Total
3	Treated	6.86	2.92	20.91	30.69
	Control	7.80	6.80	39.87	54.47
6	Treated	8.46	7.70	38.08	54.24
	Control	10.22	8.85	40.25	59.32
11	Treated	11.68	9.04	42.64	63.36
	Control	11.83	7.09	37.64	46.56
16	Treated	17.50	16.70	18.02	52.22
	Control	16.55	15.82	15.78	48.15
21	Treated	15.24	9.37	15.63	40.24
	Control	14.04	10.25	10.94	35.23
25	Treated	14.12	12.50	12.24	38.86
	Control	10.49	9.14	9.73	29.36
29	Treated	9.17	8.73	67.44	85.34
	Control	9.02	10.48	76.36	95.86
37	Treated	13.17	7.21	56.45	76.83
	Control	11.94	8.48	48.92	69.34
45	Treated	11.66	8.73	46.51	66.90
	Control	11.41	10.26	56.97	78.64

the roots with stem temperatures of 16°C to 37°C. The variability in results was high over this temperature range as is shown in Tables 4 and 7. Part of this variability was undoubtedly due to the previous history and age of the various

Table 6. The first experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of carbohydrates in the stem of tomato above the jacket after 32 hours. (Avg. of 6 plants per treatment.)

		Mg carbohydrate/g fresh wt.			
Temperature, °C	Treatment	Reducing sugar	Sucrose	Starch	Total
3	Treated	18.21	10.62	35.98	64.81
	Control	16.00	7.90	32.65	56.65
6	Treated	17.30	6.25	18.14	41.69
	Control	17.56	6.73	17.92	42.21
11	Treated	19.96	7.82	30.39	58.17
	Control	21.06	6.63	31.89	59.58
16	Treated	22.31	17.66	91.95	131.92
	Control	23.74	14.12	38.68	76.54
21	Treated	19.54	16.28	112.95	148.77
	Control	19.12	16.24	116.48	151.84
25	Treated	20.45	15.44	101.42	137.31
	Control	17.94	13.52	74.89	106.35
29	Treated	16.56	6.86	52.17	75.59
	Control	16.48	8.59	61.17	86.24
37	Treated	22.36	11.62	43.09	77.07
	Control	21.05	11.53	66.47	99.05
45	Treated	12.26	21.47	30.03	63.76
	Control	15.32	18.44	27.26	61.02

lots of greenhouse-grown plants.

It is obvious from Figure 2 that maximum translocation in these experiments occurred around 16°C. This temperature is considerably lower than that (24°C - 30°C) reported by most workers (4, 22, 48). Curtis and Herty (15) however, reported

Table 7. The second experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of sugars in the roots of tomato after 32 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Mg sugars/g fresh wt.		
		Reducing sugars	Sucrose	Total
0.5	Treated	2.32	0.06	2.38
	Control	2.45	2.14	4.59
6	Treated	4.77	2.77	7.54
	Control	4.20	3.39	7.59
11	Treated	3.65	4.14	7.79
	Control	3.81	3.85	7.66
16	Treated	4.68	3.73	8.41
	Control	4.30	4.08	8.38
21	Treated	4.13	2.91	7.04
	Control	4.85	3.93	8.78
25	Treated	4.67	2.99	7.66
	Control	5.20	3.66	8.86
29	Treated	3.30	2.76	6.06
	Control	3.41	2.84	6.25
37	Treated	4.23	4.05	8.28
	Control	4.30	4.04	8.34
45	Treated	4.16	2.82	6.98
	Control	7.28	2.39	9.67

maximum sugar movement from bean leaves at 20°C. It is felt that the low light intensity available in these experiments was the cause of the maximum occurring at such a low temperature.

Figure 2 also shows the effect of stem temperature on the

Table 8. The second experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of sugars in the stem of tomato covered by the jacket after 32 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Mg sugars/g fresh wt. Reducing sugars	Sucrose	Total
6	Treated	8.48	5.01	13.49
	Control	8.13	5.40	13.53
11	Treated	6.98	5.04	12.02
	Control	7.03	4.29	11.32
16	Treated	7.59	4.37	11.96
	Control	7.04	4.97	12.01
21	Treated	11.38	6.39	17.77
	Control	9.72	4.91	14.63
25	Treated	12.08	6.28	18.36
	Control	11.89	8.34	20.23
29	Treated	8.76	5.90	14.66
	Control	4.73	2.85	7.58
37	Treated	7.98	5.55	13.53
	Control	7.52	4.37	11.89
45	Treated	12.01	6.43	18.44
	Control	14.87	12.57	27.44

sugar concentration in the stem section covered by the jacket. Maximum and minimum accumulations occurred at the same temperatures as for root sugars. Sugar concentrations at 3°C were reduced 34 per cent below controls. At 45°C these lower stem sugar concentrations were about 80 per cent of controls.

Figure 3 attempts to show the accumulation of sugars that

Table 9. The second experiment on the effect of stem temperature, below the lowest leaf, on the accumulation of sugars in the stem of tomato above the jacket after 32 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Mg sugars/g fresh wt. Reducing sugars	Sucrose	Total
6	Treated	15.95	8.50	24.45
	Control	12.70	4.99	17.69
11	Treated	9.35	4.79	14.14
	Control	9.18	2.94	12.12
16	Treated	12.35	3.49	15.84
	Control	9.65	4.31	13.96
21	Treated	16.27	6.55	22.82
	Control	12.75	6.38	19.13
25	Treated	19.25	9.77	29.02
	Control	17.04	9.04	26.08
29	Treated	7.29	1.51	8.80
	Control	4.91	2.60	7.51
37	Treated	8.73	4.43	13.16
	Control	12.24	3.39	15.63
45	Treated	16.69	21.63	38.32
	Control	14.80	15.34	30.14

occurred above the cold jacket in the treated plants. It can be seen that at 3°C there was a considerable pile up of sugar above the jacket. The sugar concentration at this temperature was 3 times higher in the stem above the jacket than in the stem covered by it. Control plants showed a ratio of about 1.5 over all temperatures. Bonner (6) reported 3 times as

Figure 2. The effect of stem temperature, below the lowest leaf, on the total reducing sugar and sucrose content of tomato roots and of the stem covered by the jacket (lower stem), after 32 hours. The sugar content is expressed as a percentage of control plants.

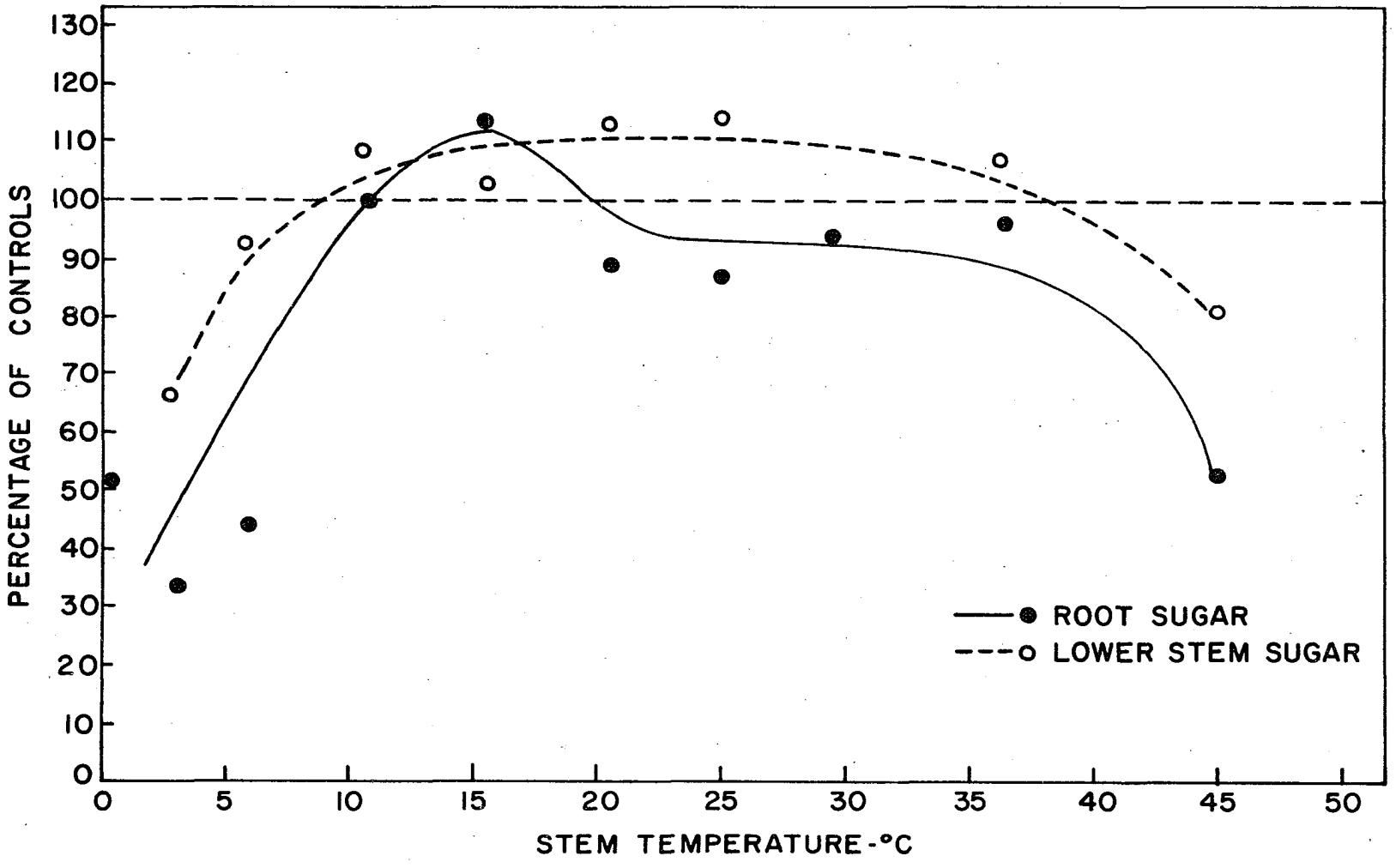
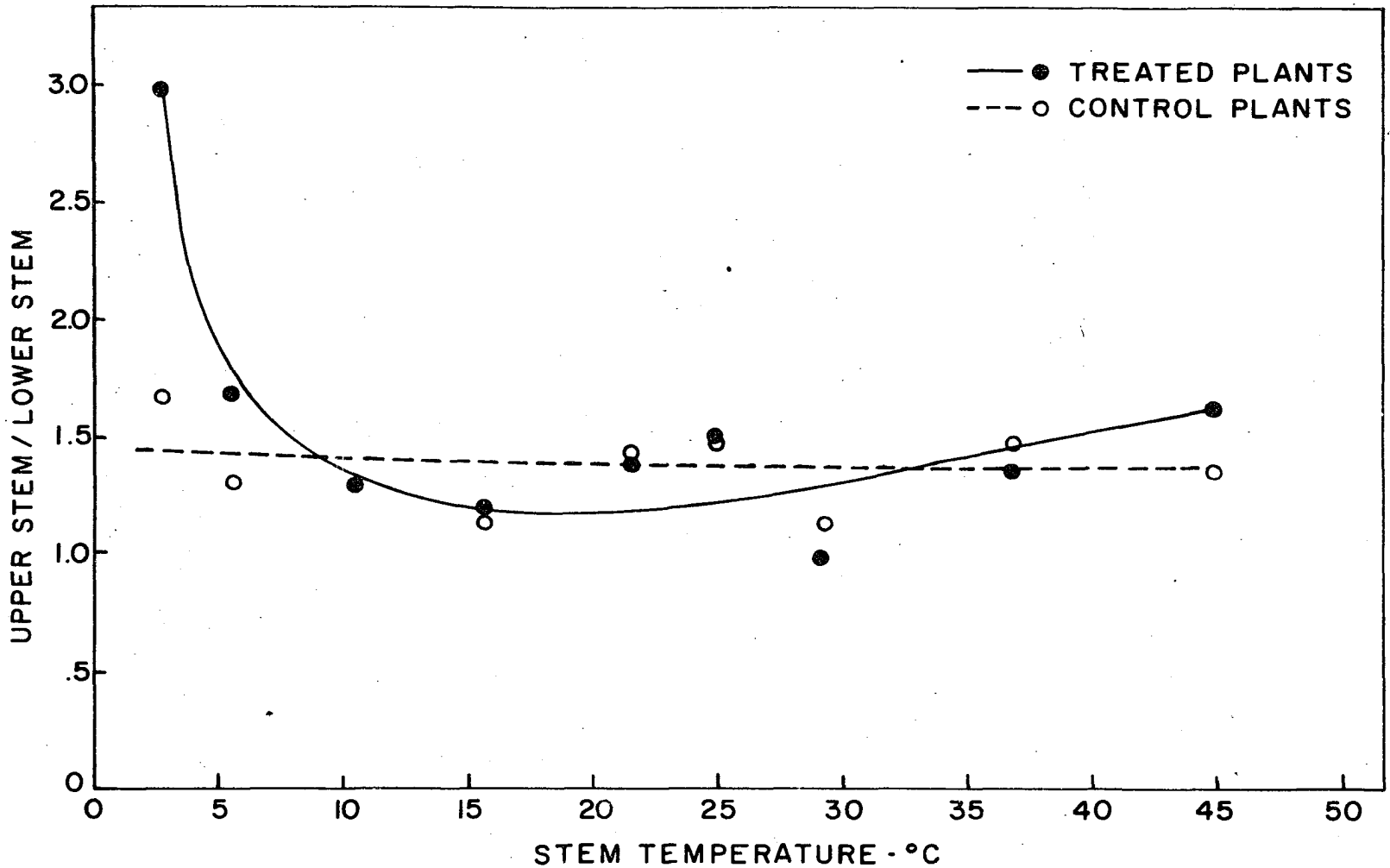

PERCENTAGE OF CONTROLS
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
STEM TEMPERATURE-°C
ROOT SUGAR
LOWER STEM SUGAR

Figure 3. The effect of stem temperature below the lowest leaf on the accumulation of total reducing sugars and sucrose in tomato after 32 hours. The accumulation is expressed as a ratio of sugar concentration in the stem sections above the jacket (upper stem) and that covered by the jacket (lower stem).


TREATED PLANTS
CONTROL PLANTS
UPPER STEM / LOWER STEM
3.0
2.5
2.0
1.5
1.0
.5
0
0
5
10
15
20
25
30
35
40
45
50
STEM TEMPERATURE - °C

much sucrose above a girdle as in the comparable stem section in control plants after 36 hours. In the present experiments the sucrose concentration above the jacket was 1.3 times that in the comparable stem section of control plants after 32 hours. As in the root data, control levels of transport were reached at about 11°C and continued rather steadily to 37°C where a slight reduction occurred. It is not known whether this increase in accumulation continues above 45°C, but it is suspected that it does.

Radioisotope Experiments

Temperature experiments

The experiments on the effects of temperature on the translocation of sugars were supplemented by experiments with the translocation of P^{32}. The phosphorus was spotted onto a terminal leaflet at the rate of 15 µc in 40 µl and the plants held as for the sugar experiments, but for 6 instead of 32 hours. The plants then were divided into appropriate fractions, dried, the material formed into briquets and counted.

The overall effects of stem temperatures below the treated leaf on the movement of P^{32} are shown in Tables 10, 11 and 12. Although the same quantity of P^{32} was used in each test, the quantity moved from the treated leaf - shown as "total" in the tables - was highly variable. A similar result has been reported by Dr. C. A. Swanson of Ohio State Univer-

Table 10. The first experiment on the effect of stem temperature, below the treated leaf, on the movement and accumulation of foliar applied P^{32} in tomato after 6 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Counts per minute				
		Roots	Stem covered by jacket	Stem above jacket	Tops	Total
0.5	Treated	35	151	464	598	1248
	Control	1191	1194	1289	1089	4763
3	Treated	281	493	1290	889	2953
	Control	2176	2264	1129	2381	7950
6	Treated	217	355	424	133	1129
	Control	763	796	734	3676	5969
11	Treated	183	239	270	215	907
	Control	396	599	408	355	1758
16	Treated	428	1522	988	444	3382
	Control	1135	1257	857	551	3800
21	Treated	318	313	221	128	980
	Control	401	595	618	247	1861
25	Treated	224	377	252	1275	2128
	Control	336	528	801	2296	3961

sity in a personal communication (1961). This variability is assumed to have been due in part to differences in the leaves of the various lots of plants used. These differences may have affected the penetration of the phosphorus and/or its movement into the phloem and out of the leaves. The very low total translocation shown by the controls of the 3°C treatment in the second series of experiments (Table 11) is, however, an

Table 11. The second experiment on the effect of stem temperature, below the treated leaf, on the movement and accumulation of foliar applied P^{32} in tomato after 6 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Counts per minute				
		Roots	Stem covered by jacket	Stem above jacket	Tops	Total
0.5	Treated	53	255	708	920	1936
	Control	1009	992	525	2920	5446
3	Treated	87	154	1045	957	2243
	Control	7	17	38	122	184
6	Treated	113	243	223	162	741
	Control	135	132	107	206	580
11	Treated	1099	591	1503	1051	4244
	Control	1382	891	1329	1089	4691
16	Treated	931	1258	784	419	3392
	Control	820	1158	723	679	3380
21	Treated	667	1143	1814	1627	5251
	Control	1308	1835	1859	1836	6838
25	Treated	519	686	850	1707	3762
	Control	779	583	1002	1792	4156

example of an unexplained variability which appears to be inherent in this type of experiment. In spite of these variations, however, a generally clear picture of the effect of stem temperature is shown by the results.

The total movement from the treated leaves of plants with temperature treated stems is shown in Figure 4. When the stem of the plants was chilled to 0.5°C, total movement was reduced

Table 12. The third experiment on the effect of stem temperature, below the treated leaf, on the movement and accumulation of foliar applied P^{32} in tomato after 6 hours. (Avg. of 6 plants per treatment.)

Temperature, °C	Treatment	Counts per minute				
		Roots	Stem covered by jacket	Stem above jacket	Tops	Total
3	Treated	6	14	239	283	542
	Control	325	375	660	417	1777
16	Treated	701	375	678	391	2145
	Control	460	258	355	374	1447
25	Treated	244	422	1414	2810	4890
	Control	75	88	315	729	1207

by 70 per cent when compared with the controls. As shown previously this effect was not due to a chilling of the leaf node or petiole of the treated leaf, but to some systemic action. Böhning et al. (4) have reported results with translocation of sugars from the leaves of bean that suggest the same effect. In their experiments, chilling the hypocotyl reduced tip growth, which was used as an index of movement from leaves above the temperature coils.

The effect of stem temperature below the treated leaf on the movement and accumulation of P^{32} in the roots of tomato is shown in Figure 5. The data are expressed in counts per minute as a percentage of control plants, and as specific activity as a percentage of control plants. Both of the

Figure 4. The effect of stem temperature, below the treated leaf, on the movement of foliar applied P^{32} out of the leaf in 6 hours. The data are expressed in counts per minute as a percentage of control plants.

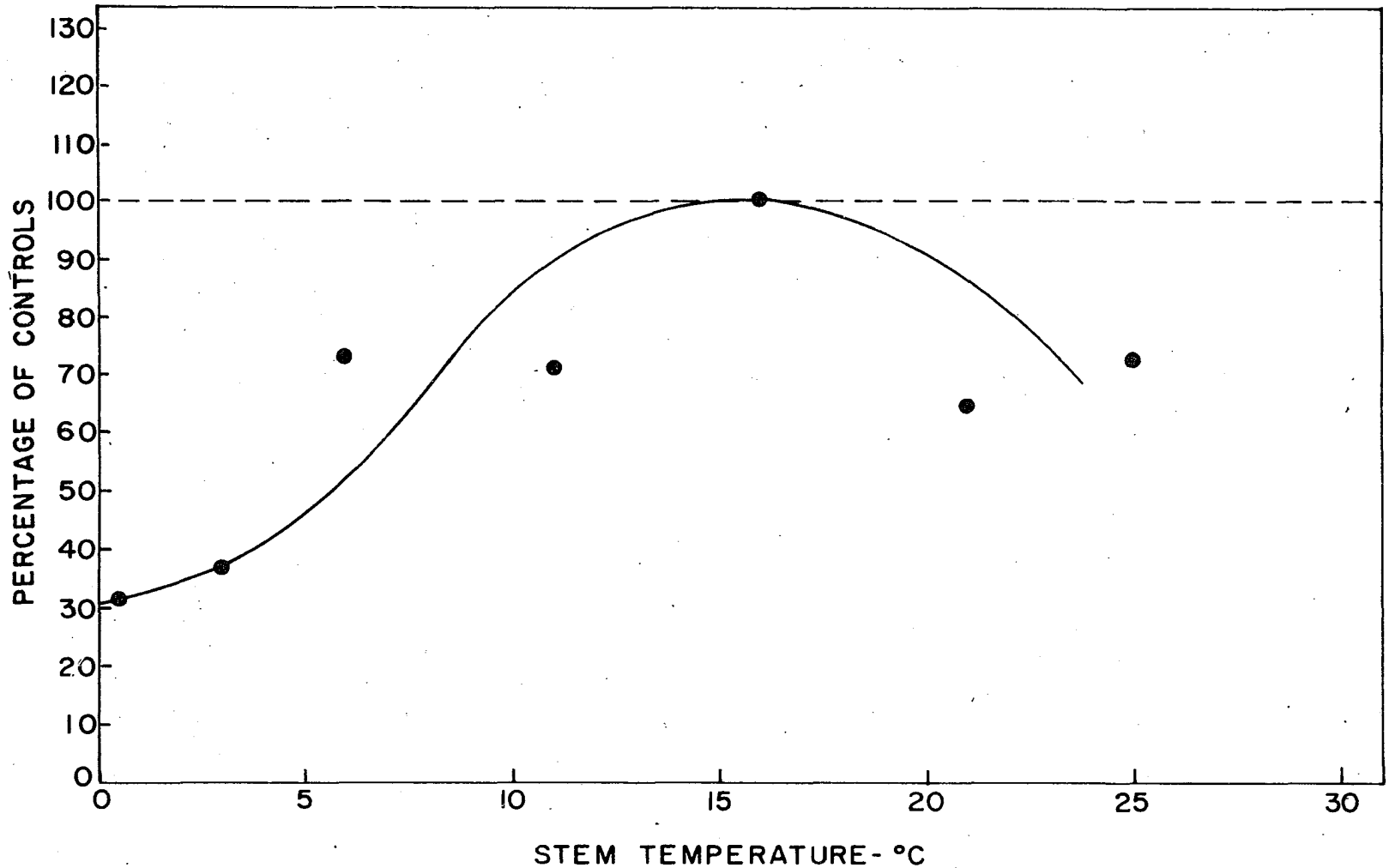
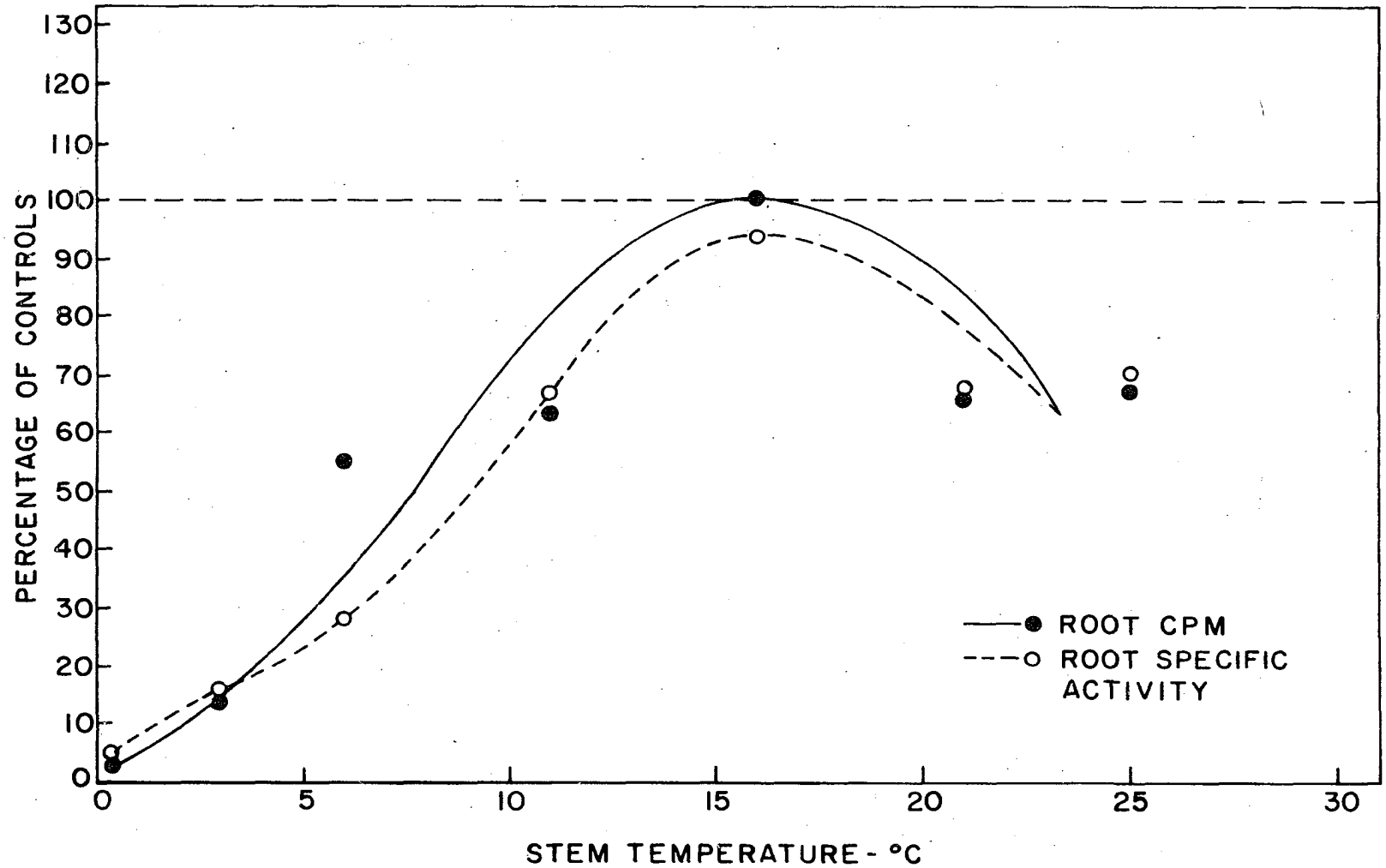

PERCENTAGE OF CONTROLS
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
STEM TEMPERATURE- °C

Figure 5. The effect of stem temperature below the treated leaf on the movement and accumulation of foliar applied P^{32} in the roots of tomato after 6 hours. The data are expressed in counts per minute and specific activity as a percentage of control plants.


PERCENTAGE OF CONTROLS
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
STEM TEMPERATURE - °C
ROOT CPM
ROOT SPECIFIC ACTIVITY

curves have the same general shape with a maximum and minima at the same temperatures. At 0.5°C, P^{32} accumulation in the roots of cold-stem plants was about 5 per cent of the controls. Transport to and accumulation in the roots increased to control levels at 16°C and decreased above this temperature to about 70 per cent of controls at 20 - 25°C. It may be noted that the temperature at which maximum movement to the roots occurred was the same as that for maximum total movement out of the treated leaf. However, since at 0.5°C total movement of P^{32} out of the leaf was 30 per cent of controls and movement to the roots was only 5 per cent of controls, it is obvious that movement out of the leaf was not the major factor which controlled movement to the roots.

For stem temperatures around 1°C, Went and Hull (52) have reported a Q_{10} for translocation of well below 1.0. Böhning et al. (5) have reported a Q_{10} for translocation of approximately 1.5 for the temperature range of 12 - 24°C. In the present experiments a Q_{10} for P^{32} transport and accumulation of around 100 was obtained for a stem temperature range between 0.5 - 3°C. From 3 - 11°C the Q_{10} was between 8 and 9, while between 11°C and 16°C a Q_{10} of nearly 2 was obtained. The Q_{10} obtained for temperatures from 11 - 16°C agrees very well with that reported by Böhning et al. (5) for 12 - 24°C.

The viscosity of water and of sucrose solutions increases at lower temperatures, and proponents of the mass flow hypoth-

esis have explained the reduction of translocation in chilled stems on this basis. The increase in the fluidity of a 20 per cent sucrose solution between 0° and 15°C shows a Q_{10} of 1.3 to 1.5. The Q_{10} of translocation over this range was about 10 in our experiments. The Q_{10} values for translocation do not support an hypothesis of physical control of the process.

Figure 6 shows the effect of stem temperature on the movement of P^{32} to the roots of tomato. The P^{32} in the roots is expressed in counts per minute and thus eliminates any consideration of the control plants in the presentation of the data. The general shape of the curve is similar to those in Figures 4 and 5 and shows a maximum and minima at the same temperatures. These results suggest that the data presented in Figures 4 and 5 are not the result of significant changes in the control plants, but are due directly of the temperature treatment.

As in Figure 3, an attempt is made in Figure 7 to show the effect of stem temperature on the accumulation of P^{32} above the cold region. In the control plants, the ratio between counts per minute in the stem above the jacket (upper stem) and in the stem covered by the jacket (lower stem) was slightly over 1 for all experiments. With a stem temperature of 0.5°C, over 7.5 times as much P^{32} accumulated above the jacket as in the stem covered by it. Control levels of transport, based on these data, occurred at 16°C. This is the

Figure 6. The effect of stem temperature, below the treated leaf, on the movement and accumulation of foliar applied P^{32} in the roots of tomato after 6 hours. Data expressed in counts per minute.

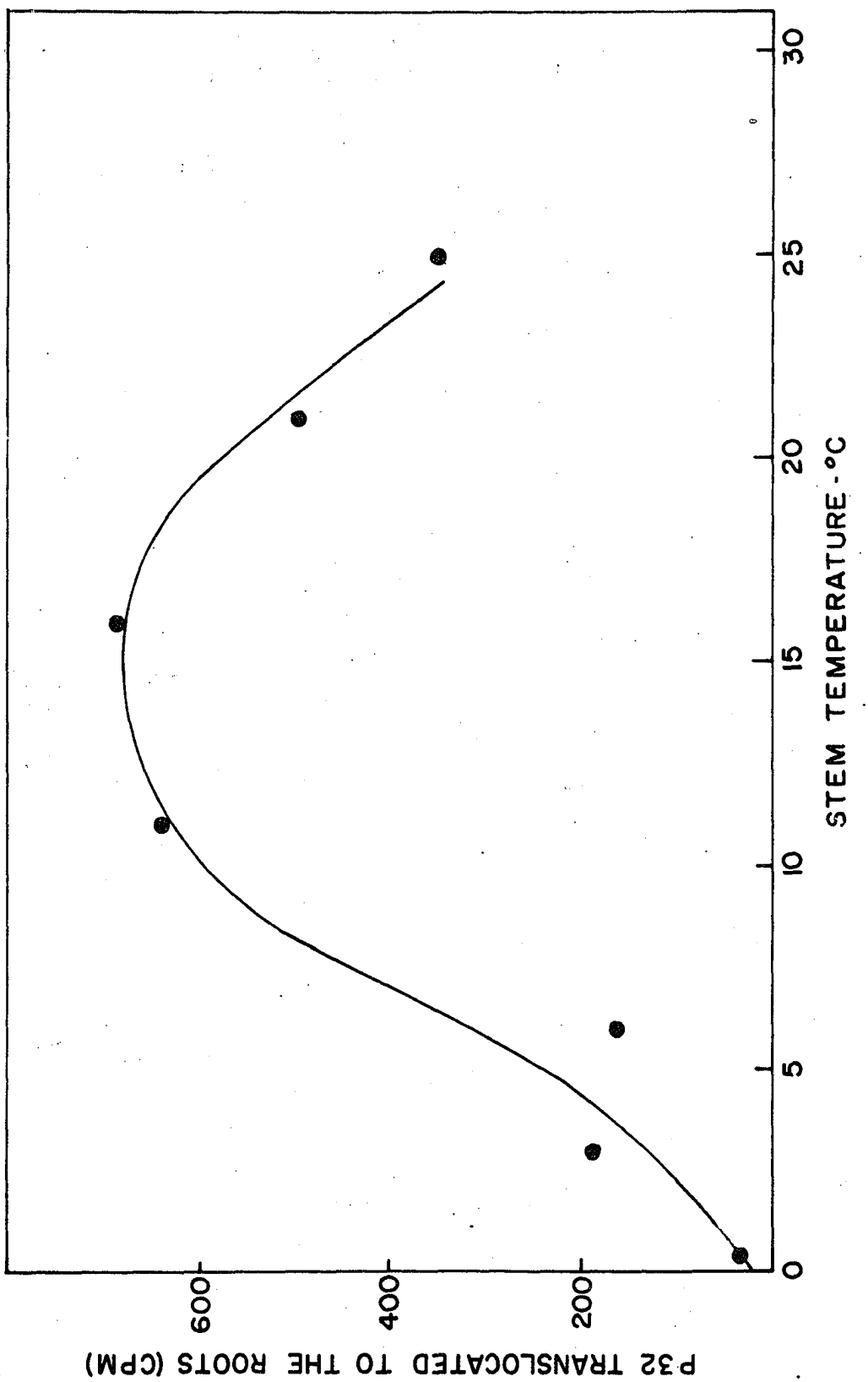
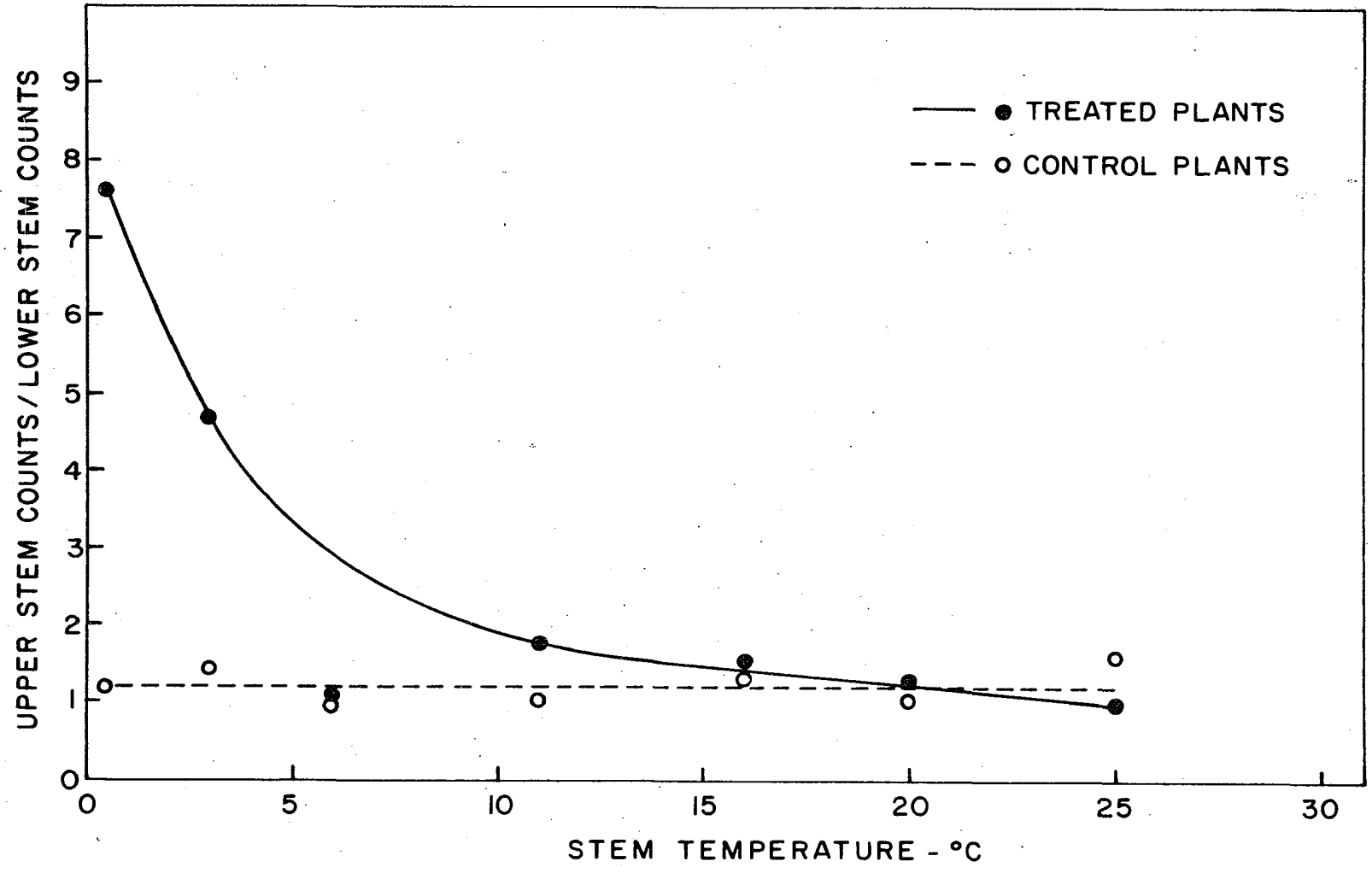

P32 TRANSLOCATED TO THE ROOTS (CPM)
200
400
600
0
5
10
15
20
25
30
STEM TEMPERATURE - °C

Figure 7. The effect of stem temperature below the treated leaf on the accumulation of foliar applied P^{32} above the jacket after 6 hours. The accumulation is expressed as a ratio of the counts per minute in the stem sections above the jacket (upper stem) and that covered by the jacket (lower stem).


UPPER STEM COUNTS / LOWER STEM COUNTS
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30
STEM TEMPERATURE - °C
TREATED PLANTS
CONTROL PLANTS

temperature at which maximum accumulation occurred in the roots. These data did not, however, indicate any inhibition of transport above 16°C, as was indicated in the root data.

The theory suggested by Böhning et al. (4) and Day (16), that interruption of the phloem at any point of the stem interferes with downward transport at all other points, appears to be untenable in the light of the results of Bonner (6) and those presented here. Since both sugars and P^{32} accumulated in the region above a cold jacket, it is obvious that all downward translocation did not stop when the phloem was interrupted. It may be that the physiological isolation of an active "sink" in the roots, and the accumulation of materials above the jacket, were the direct causes of the reduction in total movement out of the treated leaf.

Steaming experiments

A series of experiments was run in which a section of the stem, either below or above the P^{32}-treated leaf, was killed by steaming. Care was taken to insure complete killing so that no internal phloem was left active. Killing the stems constituted a more drastic interruption of the phloem than chilling, and at the same time it left the transpiration stream intact. On the assumption that downward translocation of P^{32} will normally occur in the phloem, whereas upward movement may be due to a transfer from the phloem to the xylem, it

was thought that this series of experiments would throw some light on the extent of such transfer. Because of space limitations it was not possible to make simultaneous tests of all combinations of steaming and chilling. In comparing the results of the two treatments, therefore, it should be borne in mind that the P^{32} experiments were generally made with a different lot of plants and at a different time.

The data of Table 13 show a comparison of steaming below the treated leaf with chilling below. In the steamed-stem data in Table 13 the total counts were low and the average reduction of about 20 per cent in total translocation was not statistically significant. The reduction in the movement to the roots was comparable in both series and approached the background count, indicating essentially complete interruption of downward translocation. There appeared, also, to be less accumulation above the steamed section, and a larger percentage of total movement reached the top. The data suggest transfer of P^{32} to the xylem in the steamed section, although other data, reported later, do not support this explanation.

A comparison of the effect of cooling and steaming the stem <u>above</u> a P^{32}-treated leaf is shown in Table 14. With treatments applied above the leaf there was no significant reduction in the total movement from the treated leaf with either chilling or steaming. Previous results had indicated a highly significant reduction (70%) in total movement when

Table 13. The effect of cooling (0.5°C) or steaming the stem of tomato, below the treated leaf on the movement and accumulation of foliar applied P^{32} after 6 hours. (Avg. of 12 plants per treatment.)

Plant part	Stem treatment: Cooled below — Cpm	Specific activity	Per cent of total moved	Steamed below — Cpm	Specific activity	Per cent of total moved
Tops	500 (1475)[a]	420 (1447)	43.8 (36.9)	345 (336)	229 (110)	72.6 (54.4)
Treated leaf	------					
Stem above jacket or killed segment	470 (825)	2518 (4428)	41.2 (20.6)	106 (153)	184 (228)	22.3 (24.8)
Stem covered by jacket or killed segment	140 (854)	255 (1639)	12.3 (21.4)	11 --	168 --	2.4 --
Basal stem and roots	31 (842)	48 (982)	2.7 (21.1)	13 (129)	14 (134)	2.7 (20.8)
Total	1141 (3996)	-- --	100.0 (100.0)	475 (618)	-- --	100.0 (100.0)

[a]Control values are in brackets.

Table 14. The effect of cooling (0.5°C) or steaming the stem of tomato, above the treated leaf, on the movement and accumulation of foliar applied P^{32} after 6 hours. (Avg. of 12 plants per treatment.)

Plant part	Stem treatment: Cooled above — Cpm	Cooled above — Specific activity	Cooled above — Per cent of total moved	Steamed above — Cpm	Steamed above — Specific activity	Steamed above — Per cent of total moved
Tops	47 (826)[a]	39 (710)	1.0 (14.9)	148 (317)	80 (161)	10.1 (23.6)
Stem covered by jacket or killed segment	19 (505)	73 (2071)	0.4 (9.3)	11 --	275 --	0.8 --
Stem below jacket or killed segment	101 (1415)	532 (6173)	2.2 (25.4)	20 (67)	63 (202)	1.4 (5.5)
Treated leaf	------					
Basal stem and roots	4427 (2788)	3659 (2843)	96.4 (50.4)	1281 (942)	902 (749)	87.7 (70.9)
Total	4594 (5534)	-- --	100.0 (100.0)	1460 (1326)	-- --	100.0 (100.0)

[a]Control values are in brackets.

the stem was chilled to 0.5°C below the treated leaf. As a general observation, chilling below the treated leaf had more effect on total movement of P^{32} when the roots were making relatively more growth than the tops. The roots were always pruned one week before the plants were used. The amount of root recovery varied with greenhouse growing conditions and top growth rates varied even more. It is possible that a more careful study of the effect of the balance of root growth and top growth on translocation would explain some of the apparently anomalous results that are frequently obtained in translocation experiments (2).

It is obvious from Table 14 that both cooling and killing the stem above the P^{32}-treated leaf reduced the amount of the tracer reaching the top portion of the plant. There is an indication, also, that cooling was more effective in reducing upward movement of P^{32} than killing. In the cold-stem plants, P^{32} accumulation in the tops and stem covered by the jacket was reduced to 1.4 per cent of the total moved. The comparable control plants had about 24 per cent of the activity in this portion of the plant. Steaming the stem above the leaf reduced the activity in the tops to 10 per cent of the total while controls had almost 24 per cent of their total in the tops.

Several workers (2, 3, 25, 26, 35) have suggested that there is considerable movement of nutrients in plants from

phloem to xylem tissue. The results of the present experiments indicate that such was not the case, at least over a 6 hour period. There was considerably more activity in the roots of the cold-stem plants, but considerably lower activity in the tops. This suggests that there was little or no movement of P^{32} from phloem to xylem in the roots and stem below the cold jacket in the treated plants. If lateral movement into the xylem had occurred, the P^{32} would have been swept to the tops and they would have shown greater activity.

In plants steamed above the treated leaf, there was an increase in activity in the roots, over the controls. This increase was not as great as in the cold-stem plants. Also, the tops showed a greater activity than in the cold-stem plants. There is a suggestion that lateral movement of P^{32} into the xylem occurred at the killed section and not in the roots.

It is interesting to note that there was no accumulation below either the cold jacket or the killed section such as was obtained in the "cooled below" experiments.

Four experiments were run on the effects of simultaneous cooling above and steaming below the treated leaf. In one experiment a considerable translocation of P^{32} to the roots past a steamed section indicated that the internal phloem of the tomato stem had not been completely destroyed. In two others the total counts were too low for evaluation. It is

possible that the activity of the P^{32} was incorrectly estimated, or that cold, dark weather had reduced the activity of the plants.

Plants for the fourth experiment were held in a growth chamber with controlled temperature and 3000 to 4000 foot-candles of illumination. Total counts and translocation were relatively good in these plants, and the effects of treatments were clear cut (Table 15).

The marked tendency for downward movement of P^{32} in all treatment lots is believed to be evidence of a polar movement (30) toward the roots. It is assumed that this polarization was brought about by the heavy pruning of the roots a week before the experiment was run, and the subsequent rapid regrowth of the roots. Of the total translocation, 74 per cent was downward in the control plants; 72 per cent moved downward in the steamed-below plants, in spite of the essentially complete interruption of movement to the roots. If downward movement was due to a "sink" in the regrowing root system, cutting off the sink did not change the quantity or direction of movement. Cooling above interrupted movement to the tops, but did not result in any accumulation in the stem above the treated leaf and below the cooling jacket. Downwardly directed movement was 85 per cent, again in the absence of a "sink".

One of the reasons for starting these experiments was to

Table 15. The fourth experiment on the effect of a cooled stem (0.5°C) above and steaming below the treated leaf on the movement and accumulation of foliar applied P^{32} after 6 hours. (Avg. of 3 plants per treatment.)

Plant part	Control			Stem treatment: Steamed below			Cooled above-steamed below		
	Cpm	Specific activity	Per cent of total moved	Cpm	Specific activity	Per cent of total moved	Cpm	Specific activity	Per cent of total moved
Tops	62	42	12.4	89	71	10.2	22	16	1.6
Stem covered by jacket	32	78	6.4	45	155	5.2	16	53	1.2
Stem between jacket and leaf	34	148	6.8	109	495	12.4	46	768	10.6
Treated leaf	-----								
Stem between leaf and killed section	156	264	31.2	626	1361	71.3	1169	2386	85.2
Killed section	--	--	--	3	60	0.3	11	183	0.8
Basal stem and roots	216	230	43.2	6	9	0.6	8	13	0.6
Total	500	--	100.0	878	--	100.0	1372	--	100.0

determine whether upward movement of P^{32} was in the phloem, or in the transpiration stream, after transfer from the phloem to the xylem. The results with the control plants in Table 15 show that 19 per cent of the total translocation was upward, above the cooling region. Chilling this region reduced upward movement to 3 per cent. It may be noted that the P^{32} content of the stem between the treated leaf and the cooling region was not affected by any of the treatments. The data support the conclusion that upward movement was occurring in the phloem in spite of the strong downward polarization of movement, and that no significant transfer from phloem to xylem occurred. We interpret these results as indicating that the P^{32} was moving in an organic form (29) in these plants.

DISCUSSION

It is generally accepted by plant physiologists that low temperatures inhibit translocation in the phloem. Went and Hull (52) and Went (51) have concluded from their experiments however, that low stem temperatures increased translocation of sucrose to the roots of tomato plants. In their work, Went and Hull (52) used the rate of bleeding from the cut stem above two sucrose fed leaves as an index of translocation. It was assumed that bleeding was due to root pressure and proportional to the sugar concentration of the roots. It has been shown by other workers that bleeding can be due to local solute accumulations. The hypothesis that the increased bleeding obtained in their experiments was due to decreased translocation and the accumulation of sugars above the cooling jacket is supported by their analyses. They reported that the concentrations of sucrose in the roots of the chilled-stem plants were lower than in the control plants. Went (51) held whole tomato plants overnight at temperatures of 26.5°C and 18°C. In the morning it was found that the sucrose concentrations in the leaves of plants at 26.5°C was higher than that in plants at 18°C. Went concluded that an increased rate of translocation at the lower temperature was responsible for the differences. Since translocation as such was not measured, it is not possible to determine whether 26.5°C was above the

optimum temperature for translocation, as in some of our experiments, or whether other metabolic effects were involved. Data from other workers, particularly Curtis (15) and Swanson and others (4, 48), agree in indicating that translocation is increased by rising temperatures over the range from low to moderate.

In the present experiments, in which the plants and method of stem-temperature control were similar to those of Went and Hull, a stem temperature of 0.5°C reduced the sugar concentration of tomato roots to 50 per cent of control plants after 32 hours (Figure 2). The same treatment reduced the movement of foliarly applied P^{32} to the roots to about 5 per cent of controls after 6 hours, as shown in Figure 5. Translocation of both endogenous sugars and P^{32} increased rapidly to control levels as the basal stem temperature was increased from 0.5°C to about 16°C. Sugar transport to the roots was inhibited by stem temperatures above 37°C while P^{32} movement was inhibited at temperatures above 16°C. Went and Hull (52) reported Q_{10}'s for translocation of well below 1 for the temperature range of 1 - 3°C. Böhning et al. (5) reported a Q_{10} for carbohydrate translocation of 1.5 for temperatures between 12°C and 24°C. In the present work a Q_{10} for P^{32} transport and accumulation of about 100 was calculated for a stem temperature range between 0.5°C and 3°C. From 3 - 11°C the Q_{10} was between 8 and 9, while between 11°C and 16°C a Q_{10} of

nearly 2 was obtained. The latter Q_{10} agrees well with that reported by Böhning et al. (5). The proponents of the mass flow hypothesis of Münch (40) have attempted to explain the reduction in translocation in chilled stems on the basis of increases in the viscosity of water and sucrose solutions. The decrease in the fluidity of a 20 per cent sucrose solution between 15°C and 0°C shows a Q_{10} of 1.3 to 1.5. The Q_{10} for translocation over this range was about 10 in the present experiments. These results do not support an hypothesis which suggests a physical control of the process of translocation. It is thought that phloem transport is an energy requiring process and that low temperatures reduce the available energy by decreasing the metabolic activities of the phloem cells (14, 17, 43).

It has been suggested that inorganic materials may circulate within a plant (2, 3, 10, 26, 35). This circulation is dependent upon downward movement of these inorganic materials in the phloem, radial diffusion into the xylem and upward movement in the latter tissue. Loomis (29) has suggested that such a circulation does not occur with organic materials. Norman G. Sansing, Graduate Student, Iowa State University, has reported in a personal communication (1961) that steaming the stem of soybean above a P^{32} treated leaf did not reduce the amount of the tracer reaching the top of the plant. Simultaneous steaming of the stem above and below the leaf

also had no effect on P^{32} activity in the top of the plant. Steaming the stem below the treated leaf caused activity in the top to increase greatly over the controls. The results suggest that the P^{32} was being translocated in an inorganic form, and radial movement from phloem to xylem was occurring.

In contrast to these results, in the present experiments with tomato, P^{32} activity in the tops of the plants was reduced to 1.6 per cent of the total when the stem was simultaneously cooled above and steamed below the treated leaf. There was a smaller reduction when the stem was steamed above. When the stem was steamed below the treated leaf, P^{32} movement to the tops was not affected. The results indicate that the P^{32} in the tomato plants was being moved in an organic form and that movement in soybean in contrast was predominantly in the inorganic form. Upward movement of organic phosphorus in the phloem would account for the low top activity obtained when the stems were cooled above the treated leaf.

It has been suggested (29, 30), that materials being transported in the phloem exhibit polarity in the direction of their movement. Loomis (30) has shown that sugar translocation in maize is polarized out of the leaf toward the developing fruit. This polarity was established after pollination in the early phases of embryo development. Biddulph and Markle (2) have reported that foliar P^{32} moved out of a cotton leaf

in the phloem and moved upward in amounts ranging from 0 to 40 per cent of the total moved. This variability in upward movement can be explained on the basis of the growth balance between the tops and roots of the plants. The higher the metabolic activity in the tops relative to the roots, the greater the percentage of P^{32} which would move upward.

The radioisotope data reported in the present work indicate that P^{32} movement out of tomato leaves exhibited a variable downward polarity toward the roots. This polarity was apparently the result of the balance between root and top growth. The balance in most plants was tipped toward the roots in our experiments by the practice of pruning the roots heavily one week before the plants were used. If this downward movement was due to a "sink" in the actively regrowing roots, the physiological isolation of this sink did not immediately change the direction of movement. It can be seen in Figure 5 that a large accumulation of P^{32} occurred above a 0.5°C stem segment. The intensity of the accumulation decreased as the stem temperature increased from 0.5°C to 16°C. Since both sugars (Figure 3) and P^{32} accumulated above a cold jacket, it is obvious that the removal of the sink in the roots with low temperatures did not change the direction of movement after 12 hours. Apparently once a polarity of movement has been extablished in the direction of a metabolically active site, the movement tends to continue even after isola-

tion of the active site. Our data offer some suggestion of the time over which polarization may be expected to persist. The data of Figure 3 show that an accumulation of sugars was present above a cold jacket 32 hours after application of the jacket. One week after application of the jacket, the sugar accumulation above the jacket was still present but somewhat reduced (Table 2).

The suggestion discussed earlier (4, 16) that interruption of the phloem at any point interferes with the downward movement at all other points may be explained on the basis of the results presented here and those of other workers (6, 33). The present data suggest that once the direction of transport has been established, interception of the phloem has little immediate effect on the direction of translocation. The data given in Figure 4 show that total movement of P^{32} out of the treated leaf was reduced to 30 per cent of controls by a basal stem temperature of 0.5°C. The data of Table 14 show that interrupting the phloem above the treated leaf had no effect on translocation from the leaf. It is assumed that these differences were due to a downward polarization of movement in our plants so that interruption of downward translocation caused a backing up of the movement and a reduction in movement from the leaf. If fruiting plants had been used, with a polarization of translocation toward growing fruits, we might expect that interruption above the leaf would have given the

reduced transport obtained here by interruption below. As discussed earlier, the accumulation of sugar and P^{32} above a low interruption, but not below a high one, supports this explanation.

It is evident from consideration of the literature and from the results of the present experiments that phloem translocation is not a simple process. Neither the physical model proposed by Münch (40), nor the cytoplasmic streaming theory as first suggested by De Vries (17) will completely explain all of the observations which have been made on translocation. The bulk of the evidence suggests that a complicated mechanism is controlling translocation. The temperature data reported here indicate that this mechanism is limited at some point by chemical processes.

SUMMARY

The temperature of a four-inch area of the stems of tomato plants was varied by circulating water through glass jackets fitted around the stems. The roots of the experimental plants were pruned heavily before the tests to insure a translocation gradient to the regrowing root system.

The movement of endogenous sugars to the roots was reduced significantly by temperatures below 10°C or above 35°C. Differences at intermediate temperatures were variable and not statistically significant.

Where P^{32} was applied to the surface of a mature leaf the tracer was moved throughout the plant. Movement, both upward and downward, was essentially prevented by a stem temperature of 0.5°C. Movement increased with rising temperature to 16°C. The Q_{10} between 0.5 and 3.0°C was more than 100, and that between 0.5 and 16°C was 10. These values indicate that translocation was controlled at some point by a chemical reaction or reactions.

When the tomato stem was chilled to 0.5°C below the treated leaf, total movement from the leaf was reduced by 70 per cent, and the tracer accumulated above the cold jacket. When the jacket was placed above the treated leaf there was no reduction in total movement and no accumulation below the chilled section.

Because of the root-pruning treatment used, about 70 per cent of the total movement of P^{32} was downward toward the roots. The accumulation above a low jacket is considered to be evidence for a polarization of movement toward the roots, which continued even after the "sink" in the roots was isolated by the chilling. The reduction in movement from the treated leaf by the low jacket is considered to have been due to the blocking of this polarly downward movement and not to any general effect on the phloem. A cold jacket above the treated leaf did not show these effects, even though it did stop upward movement.

The inhibition of upward as well as downward movement by chilling the stem is evidence that translocation of P^{32} in these experiments was in the phloem. When the upper stem section was killed by steaming, however, the P^{32} moved upward. Additional work is needed to determine whether this difference was due to changes in permeability or to changes in the form in which the P^{32} was moving.

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